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Plant-Mediated Effects on Pymetrozine Efficacy against Aphids

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Abstract: The influence of plant factors on the efficacy of pymetrozine (CGA 215 944), an insecticide against homopteran pests, was investigated. Among all parameters tested, plant species was found to influence pymetrozine activity most. Differences in the compartmentation of pymetrozine within tissues of different plant species were found: 33% of extractable compound was measured in the intercellular space of tomato leaves, but only 5% in the same compartment of sugar beet leaves. Foliar uptake after spray application was around 5% in both plant species under a variety of conditions. The persistence of foliar-applied pymetrozine is comparable on tomato and on sugar beet plants. Following drench application, efficacy correlated with plant age and leaf development stage, but not with soil type. In conclusion, the variations in pymetrozine efficacy observed on tomato, sugar beet and pea against *Myzus persicae* (Sulzer) and *Aphis craccivora* (Koch) may be attributed to differences of pymetrozine distribution within leaf tissues and/or host plant specific feeding behaviour of aphids.

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Key words: Insecticide, pymetrozine, CGA 215944, leaf penetration, intercellular wash fluids (ICF), tomato, sugar beet, pea

1 INTRODUCTION

In the development of xenobiotics for commercial use, plant systemic effects are favoured because targeting is improved at lower application rates of the active ingredient, thereby reducing the environmental impact. Parameters considered to be very important for the systemic activity of compounds are uptake and translocation rates. Uptake occurs either *via* the shoot after foliar application or *via* the root after drench application. Once inside the plant compounds can be translocated in the vascular tissue, in the xylem and phloem. From uptake and translocation studies, models have been developed to predict the cuticular penetration and translocation potentials for compounds based on their physicochemical properties.[1–3] In addition, surfactants and adjuvants have been developed to enhance penetration, and molecular approaches to compound design have been used to improve overall phloem mobility.[3–7] However, to achieve high systemic efficacy, xenobiotics have to reach the feeding site of target insects. This may be a crucial point for the control of plant-sucking pests, which make contact with defined plant compartments only. In this case, the efficacy of a compound may not be determined only by the translocation rate but also by the compartmentation within plant tissues. In the present work this hypothesis has been investigated with pymetrozine (CGA 215 944), a selective insecticide against aphids and whiteflies.[8] This pyridine azomethine is a feeding inhibitor. It acts most efficiently by ingestion and less through contact after topical application.[9] It is translocated in the xylem and phloem leading to a plant systemic effect.[10] To investigate plant-mediated effects on the activity of pymetrozine, dose–mortality assays were performed under various conditions against *Aphis craccivora* (Koch) and

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Myzus persicae (Sulzer) on sugar beet plants, *Beta vulgaris* L. *ssp. altissima*, tomato plants, *Lycopersicum esculentum* Mill., and pea seedlings, *Pisum sativum* L. Plant penetration and intercellular localization were studied using radiolabelled compound.

2 MATERIALS AND METHODS

2.1 Bioassays

2.1.1 Insects

Insects were reared in growth chambers. Populations of *M. persicae* were reared on pea seedlings (var. Petite Provence from Fenaco, Basel, Switzerland) at 20°C, 15 : 9 h light : dark, and of *A. craccivora* on broadbean seedlings (*Vicia faba* L. var. Witkiens from Samen Mauser, Winterthur, Switzerland) at 19°C, 15 : 9 h light : dark. Strains with a normal insecticide sensitivity were used.

2.1.2 Plants

Tomato (var. Marmande from Samen Mauser, Winterthur, Switzerland) and sugar beet plants (var. Reka from FR Strube, Schoeningen, Germany) were grown in pots in the greenhouse at 21°C (14 : 10 h day : night cycles) either in peat, consisting of commercially available sphagnum peat + sand (80 + 20 by volume; pH(H_2O) 6·7, organic matter $>0{\cdot}7$ m^3 m^{-3}), or in sterilized sandy loam (pH(H_2O) 7·7; organic matter 0·026 m^3 m^{-3}; $CaCO_3$ 5 mg g^{-1}; clay 0·283 m^3 m^{-3}; silt 0·35 m^3 m^{-3} and sand 0·341 m^3 m^{-3}). Pea seeds germinated on a grid at 100% RH.

2.1.3 Application

Pymetrozine (CGA 215 944; Fig. 1) was used as a 250 g kg^{-1} WP ('Chess'; 'Plenum').

For the experiments, three different applications were performed with the product diluted in water:

I. Whole plants were treated in a spray application box using 100 ml of diluted product per 3 plants until runoff.

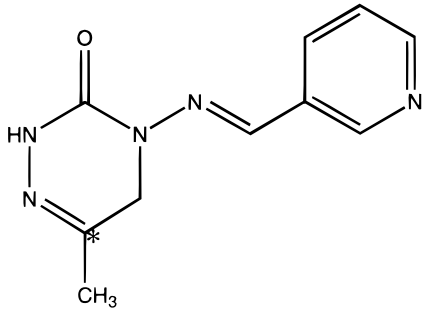

Fig. 1. Pymetrozine: 4,5-dihydro-6-methyl-4-(3-pyridylmethyleneamino)-1,2,4-triazin-3(2*H*)-one with indication of the ^{14}C position (*) of the radiolabelled compound.

II. Pot soils were drenched. Concentrations are expressed in weight of active ingredient per unit soil volume.

III. Pea seedlings were kept in pymetrozine solutions. Concentrations are expressed as weight of active ingredient per unit volume of solution.

2.1.4 Bioassay

(a) Agar (20 g $litre^{-1}$, 2 ml or 5 ml) was poured into Petri dishes (3·5 cm or 5 cm diam. respectively). The solidified agar was covered with fresh liquid agar (10–20 g $litre^{-1}$) and punched leaf discs of tomato (3·5 cm diam) or sugar beet (5 cm diam.) were gently placed on the top with adaxial surface (upper side) towards the agar. Unsynchronized populations of aphids were brushed from the appropriate rearing substrate onto leaf discs. Petri dishes were covered with cotton filters and closed with tight-fitting plastic lids. They were kept in a growth chamber (20°C, 12 : 12 h day : night cycles, 60% RH) with the upper leaf side exposed to the light. The virginoparae produced nymphs overnight. Subsequently, the virginoparae, exuviae and excess nymphs were removed using a vacuum pipe so that 25–40 nymphs remained on each leaf disc.

(b) Pea seedlings were brought into contact with heavily colonized rearing plants at six days after germination. One day after infestation, seedlings were inserted with their roots through the hole of a plastic lid into 25-ml glass vials containing pymetrozine solutions.

2.1.5 Mortality assessment and statistical analysis

Mortality rates in bioassays were assessed five days after infestation of sugar beet and tomato leaf discs and seven days after application to pea seedlings. Nine replicates—three plants and three leaf discs per plant or nine seedlings respectively—were used per experiment and concentration. All experiments were repeated at least twice. Each assay included a control consisting of water treatment. Control mortality rarely exceeded 15% for both aphid species. Results were subjected to probit analysis using SAS software (SAS Institute, Cary, NC) for the estimation of LC_{50} values and 95% fiducial limits. Influence of plant species, plant age, leaf development stage and soil type was tested at a significance level of 5%.

2.2 Uptake rates and intercellular wash fluids (ICF)

2.2.1 Plants

Tomato and sugar beet plants were grown on peat under the same conditions as described in Section 2.1.2. For comparison, outdoor-grown tomato plants were included in the experiments. Fully expanded leaflets of 4 to 8-week-old tomato plants or leaves of 1 to 15-week-

old sugar beet plants were treated with radiolabelled compound.

2.2.2 Application

[*Triazine-6-*^{14}C]pymetrozine with an activity of 2·02 Mbq mg^{-1} and with a >95% radiochemical purity was used. The radiolabelled compound was formulated as 250 g kg^{-1} WP by grinding the labelled compound and the blank formulation (1 + 3 weight, 10 min) in an Esco Typ 50/35 mortar (Esco Labor, Riehen, Switzerland).

Uptake studies were performed under various conditions on tomato plants after spray application. To compare penetration rates and intercellular wash fluids (ICF) of tomato and sugar beet plants, droplets were applied.

I. Spray application: Either the upper or under sides of tomato leaflets were sprayed until run-off with 500 μl of a WP dispersion containing 150 ng μl^{-1} pymetrozine. Spraying was performed in a spray application box with a thin layer chromatography (TLC) jet connected to a hand pump. The adjacent plant parts of the treated leaflet were protected with aluminium foil.

II. Droplet application: For sugar beet plants, fully expanded leaves were placed between two pieces of card (10·5 × 7·5 cm). A hole (5 cm diam.) was punched into the cardboard facing the upper leaf side. The cards were clamped together and the leaves were aligned horizontally on support devices. On the exposed leaf disc, small droplets of a 150 ng AI μl^{-1} dispersion were pipetted up to a total volume of 300 μl. For tomato plants, leaflets were fixed in Petri dishes (5 cm diam.) and held in a horizontal position by support devices. On the upper leaflet side, small droplets of a 150 ng AI μl^{-1} dispersion were pipetted, with a total volume of 300 μl per leaf.

2.2.3 Preparation of intercellular wash fluids (ICF), homogenates depleted of intercellular fluid and total homogenates

A slight modification of the protocol according to De Wit and Spikeman[11] was used: treated leaflets were excised from tomato plants and treated leaf discs were punched from sugar beet leaves. The deposit on the leaf surface was washed off by dipping the treated leaf material in water + methanol (1 + 1 by volume, 30 ml; 2 × 30 s). Subsequently, the leaf material was washed in water (30 s), dried between filter paper, gently rolled, introduced into 15-ml tubes, infiltrated under vacuum with water and blotted dry with filter paper. It was rolled again, introduced into plastic pipette tips (5 ml) sealed with glass fibre filters and centrifuged (900*g;* 30 min) in 15-ml tubes. The ICF eluted was collected into 500-μl Eppendorf tubes placed at the bottom of the centrifugation tubes. The leaf material was then homogenized in an Omni mixer (30 s) with methanol (30 ml) and extracted with methanol (30 ml; 2 × 30 min) on a shaker; extracts were centrifuged (4000*g;* 5 min) and the supernatants were combined after filtration to obtain ICF-depleted homogenates. Non-extractable radioactivity was determined by combustion of the dried extracted material. Total homogenates were prepared from treated leaf material, after washing off the deposit, using the same procedure as described above.

Radioactivity was measured with a Canberra-Packard Tri-Carb Scintillation Counter Model 2200CA (Packard Instruments Comp. Inc., Downers Grove Ill., USA). Oxysolve C-400 (Zinsser Analytik GmbH, Frankfurt, FRG) was used as counting cocktail. Quenching was corrected for by the tSIE method (transformed Spectral Index of External standard spectrum by reverse spectral transformation, Packard Instr.).

2.2.4 Measurement of hexose-6-phosphate isomerase

In parallel samples, the intracellular marker hexose-6-phosphate isomerase was measured[12] in the ICF and in the homogenates depleted of intercellular fluid. In contrast to the procedure above, plant material was homogenized with liquid nitrogen in a mortar and pestle and extracted in sodium borate (0·1 M; 500 mg litre^{-1}; pH 7·8). The extracts were centrifuged (12 000*g*) and enzyme activity was measured in the supernatant.

2.2.5 Evaluation and statistical analysis

Penetration rates and ICFs were assessed on triplicate samples. All experiments were repeated at least twice. Penetration rates were related to plant species, leaf side, incubation time, growth condition (glasshouse or outdoor) and dew simulation in the GLM procedure of SAS system (SAS Institute, Cary, NC) (significance level 5%). The pymetrozine relation of ICF to ICF-depleted homogenates in tomato and sugar beet plants was compared in a single factor analysis of variance (ANOVA) at a significance level of 5%.

3 RESULTS

3.1 Bioassays

Differences in pymetrozine activity were found against *A. craccivora* and *M. persicae* (Table 1). On both tomato and pea, ten times higher concentrations were required to control *A. craccivora* than *M. persicae*. Furthermore, large differences in pymetrozine activity were found, not only against different aphid species but also against the same aphid species feeding on different plant species. On sugar beet, 40 times the concentration was needed to achieve the same effect by foliar application against *M. persicae* than on tomato plants (Table 1). However, no significant effect was caused by plant species against *A. craccivora:* the LC_{50} values were

TABLE 1
Activity of Pymetrozine against Two Aphid Species on Various Plants

Plant	Application	Bioassay on	Myzus persicae LC_{50}(mg litre^{-}) Conc.	Myzus persicae 95% FL	Aphis craccivora LC_{50}(mg litre^{-}) Conc.	Aphis craccivora 95% FL
Tomato	Foliar	Leaflet discs	0·3	0·2–0·4	—	—
Sugar beet	Foliar	Discs of expanded leaves	—	—	3·8	2·3–5·9
Sugar beet	Foliar	Discs of emerging leaves	12·9	8·3–20·4	3·0	1·7–4·2
Pea	In liquid medium	Seedlings	0·2	0·2–0·3	2·6	2·3–2·9

Myzus persicae showed high natural mortality on expanded sugar beet leaves which prevented statistical analysis.

uniform (*c.* 3 mg litre^{-1}) on the three plants tested (Table 1). The persistence of pymetrozine against *M. persicae* and *A. craccivora* did not differ significantly between foliar-treated tomato and sugar beet plants over time. The activity decreased by a factor of 2 to 5 between 1 and 22 days after treatment (Fig. 2). As observed with foliar application, plant species had a strong effect on pymetrozine efficacy against *M. persicae* after drench application (Fig. 3A). There were significant effects of leaf development stage on mortality of *A. craccivora*, with more aphids moribund on fully expanded leaves ($P < 0·03$) (Fig. 3B). There were also significant interactions between plant age and pymetrozine concentration; plant age had no effect at 10 mg litre^{-1}, but more aphids died on 12- and 15-week-old plants at 100 mg litre^{-1} ($P < 0·01$) (Fig. 3D). The soil type did not influence mortality rates after drench application ($P > 0·1$) (Fig. 3C).

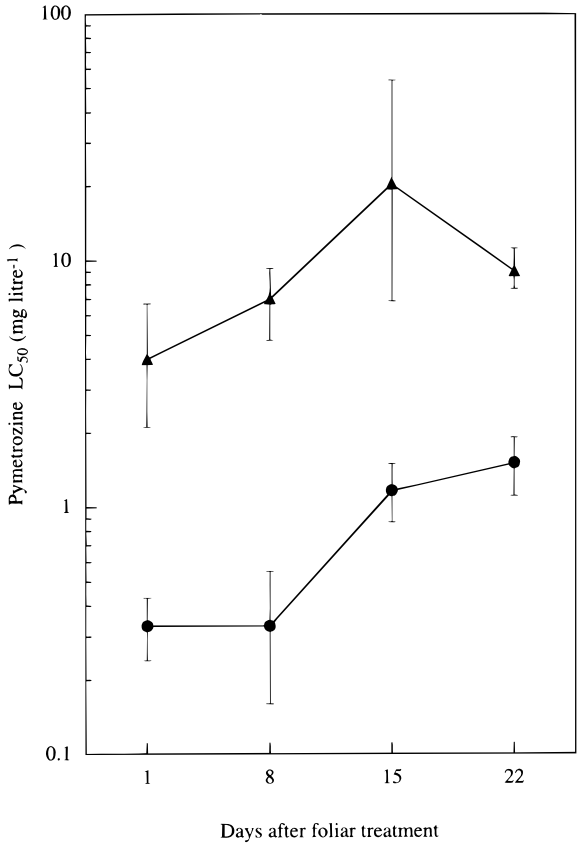

Fig. 2. Persistence of pymetrozine activity on (●) fully expanded tomato leaves against *Myzus persicae* and (▲) sugar beet leaves against *Aphis craccivora* (bars represent 95% fiducial limits).

3.2 Uptake and ICF

A maximum of 8% of total applied radioactivity was found to have penetrated into tomato leaflets after foliar treatment (Table 2). Penetration rates were not influenced by any of the parameters tested, neither by growth conditions—greenhouse or outdoor—($P > 0·9$) nor leaf side treated ($P > 0·9$), and the penetrated radioactivity did not increase between 1 and 7 DAT ($P > 0·9$) (Table 2). Furthermore, penetration was similar in both plant species; neither the percentage radioactivity taken up, nor the ratio of extractable to non-extractable radioactivity was found to be different between tomato and sugar beet plants ($P > 0·9$), but a considerable difference was found between these two plants in regard to the ICF (Table 3). In tomato leaf tissue, 33% of extractable radioactivity was found in the ICF, compared to 5% in sugar beet ($P < 0·01$). The enzyme activity of the intracellular marker hexose-P-isomerase in the ICF did not exceed 5% of total activity in either plant species (Table 3).

4 DISCUSSION

High efficacy of pymetrozine against *M. persicae* and *A. craccivora* was found on tomato and sugar beet plants one day after foliar application. With increasing time after application efficacy decreased; this was not expected, since parent pymetrozine is degraded with an apparent half-life of about three days in plants.[13] The metabolites formed are biologically inactive or very unstable (H. Szczepanski, pers. comm.). Despite this high degradation rate, the activity against the two test

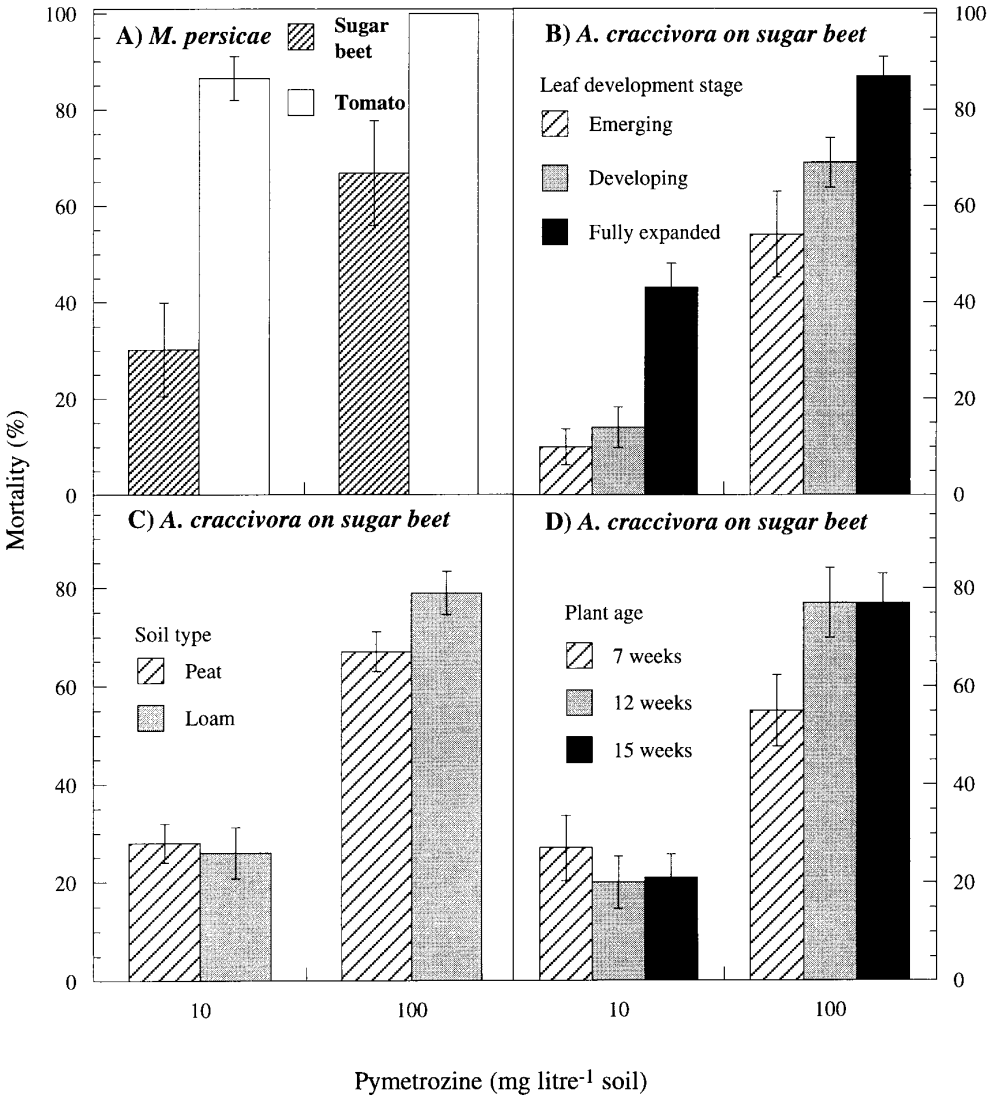

Fig. 3. Mortality rates assessed on leaf discs 15 days after drench application. A. *Myzus persicae* on tomato and sugar beet leaves. B. *Aphis craccivora* on sugar beet leaves at various development stages. Emerging leaves were not yet present and developing leaves were just emerged at time of treatment. C. *Aphis craccivora* on leaves of sugar beet plants grown in different soil types. D. *Aphis craccivora* on leaves of sugar beet differing in plant age. Plant age is reported at time of treatment (bars represent standard deviation).

insects in both foliar-treated plant species decreased only by a factor of 2–5 until 22 days after application, when 150 times less of initially penetrated parent compound is presumed to be available. This suggests that pymetrozine concentration at the food intake site of the insects does not decrease at the same rate as it degrades elsewhere. This effect could be attributed to a slower catabolic activity at the feeding site or to a continuous supply from the surrounding compartments to the feeding site. The leaf surface as supplying compartment can be excluded, since the main penetration occurs during drying of the aqueous spray deposit. Between one and seven days after foliar application of radiolabelled pymetrozine, no increase in radioactivity could be detected in the leaves.

After drench application, soil type did not influence pymetrozine efficacy. Therefore availability of the compound to the plant must be presumed to be similar in both soils. This is determined by degradation rate, soil mobility and adsorption to soil particles. Degradation of pymetrozine in soils depends mainly on biological activity and pH value. Its half-life ranges from three days in biologically active aerobic soils to 33 days in sterile aerobic soils at pH(H_2O) 7.[14] The soils used in the drench application experiments were both aerobic sterile at pH(H_2O) 6·7 and 7·7, respectively, suggesting similar degradation rates with half-lives of about one month. Therefore, degradation rates were not expected to interfere with pymetrozine availability in the soils tested. Rather, interferences were expected from differences in pymetrozine mobility and adsorption, since adsorption is reported to be highly correlated with organic matter with mean values of 300 μg g.[15] However, the results of the drench application assays

TABLE 2
Uptake of [^{14}C]Pymetrozine by Tomato and Sugar Beet Leaves after Foliar Application

	Tomato									*Sugar beet*
Growth condition	*Greenhouse*					*Outdoor*				*Greenhouse*
Leaf side treated	*Upper side*			*Under side*		*Upper side*		*Under side*		*Upper side*
Sampling (DAT)[a]	*1*	*2*	*7*	*2*	*7*	*2*	*7*	*2*	*7*	*1*
Radioactivity	(% *of initial*) (±*SD*)									
Deposit	96·3 (±1·1)	97·1 (±0·8)	96·7 (±1·3)	96·7 (±1·8)	96·2 (±0·9)	95·2 (±1·7)	92·4 (±3·1)	98·7 (±1·0)	95·0 (±1·0)	96·7 (±1·2)
Uptake	3·7 (±1·1)	2·9 (±0·8)	3·3 (±1·3)	3·4 (±1·8)	3·8 (±0·9)	4·8 (±1·7)	7·6 (±3·1)	1·3 (±1·0)	4·9 (±1·0)	3·3 (±1·2)
Extractable uptake	3·4 (±1·0)	2·7 (±0·6)	3·0 (±1·2)	3·1 (±1·7)	3·4 (±0·7)	3·3 (±0·7)	4·0 (±1·5)	0·8 (±0·8)	2·4 (±1·4)	2·9 (±1·1)
Non-extractable uptake	0·3 (±0·2)	0·2 (±0·1)	0·3 (±0·1)	0·2 (±0·0)	0·4 (±0·2)	1·5 (±1·1)	3·6 (±1·6)	0·5 (±0·2)	2·5 (±1·4)	0·5 (±0·2)

[a] Days after treatments.

TABLE 3
Radioactivity Found in the Extractable Fraction of Tomato and Sugar Beet Leaves One Day after Foliar [^{14}C]Pymetrozine Application. Percentage Separation into Intercellular Wash Fluids (ICF) and ICF-depleted Homogenates

	Tomato				*Sugar beet*			
	[^{14}C]*Pym*		*HPI activity*		[^{14}C]*Pym*		*HPI activity*	
	(%)	(±*SD*)	(%)	(±*SD*)	(%)	(±*SD*)	(%)	(±*SD*)
ICF	33·1	(±0·3)	3·5	(±1·0)	5·4	(±2·1)	1·9	(±0·6)
Homogenates (ICF-depleted)	66·9	(±0·3)	96·5	(±1·0)	94·6	(±2·1)	98·1	(±0·6)
Extractable	100·0		100·0		100·0		100·0	

Averages ot triplicate samples were taken. Hexose-P-isomerase (HPI) is an intracellular marker.

indicated that differences in adsorption and mobility, which may have occurred in the two test soils containing 5% organic matter in the sandy loam and 70% in the peat, did not interfere with the activity in the plants. In contrast, physiological age of the plant tissue revealed significant effects on pymetrozine performance after drench application: mortality on expanded sugar beet leaves was higher than on emerging leaves. In the two weeks between application and bioassay leaves emerged and developed. In such leaves, the expansion may have made it more difficult to reach the same concentration of pymetrozine compared to leaves already fully expanded at application.

Plant species had a large influence on the activity of pymetrozine against aphids. This variation in activity was not influenced by the application method. Although the results of the drench application bioassays have large confidence intervals, the activity pattern is comparable to that observed after foliar application. Accordingly, application-dependent factors, such as foliar uptake rates, were not different on the two plant species tested. Moreover foliar uptake is influenced neither by dew formation nor leaf side nor growth conditions (inside or outdoor). Translocation also seems not to be involved in the variation of activity observed against *M. persicae* and *A. craccivora* on tomato and sugar beet plants, since no differences were detectable between these two plants.[10]

However, significant differences could be observed in the distribution of pymetrozine within the leaf tissues after foliar application. In tomato plants, 33% of extractable radioactivity could be detected in the intercellular space of the leaf tissue, as opposed to 5% in sugar beet plants. This may explain some of the effects observed, since the stylet of aphids penetrates plant tissues intercellularly.[16,17] If pymetrozine is found in the intercellular space, aphids may already come into contact with the compound before they reach the final feeding site. Variation of pymetrozine distribution between tomato and sugar beet leaf tissue could therefore be an important factor in the differences in activity observed. However, this does not readily explain the additional phenomenon observed on pea seedlings: while the activity against *M. persicae* was four times lower than for *A. craccivora* on sugar beet, it was reversed by a factor of 13 on pea seedlings. This has probably to be attributed to other factors, such as differences in phloem loading, or plant-species-dependent feeding behaviour of the aphids. Adaptation of feeding behaviour to host plants seems to be an important contribution to the wide host range of polyphagous pests. For example, potato leafhoppers feed from different cell types on broad bean and on alfafa respectively.[18] Such adaptations may also have evolved in the two polyphagous aphid species used in the bioassays. This seems possible, since aphids do not feed exclusively on the phloem: in order to compensate for water deficit, as a result of losing water to the relatively concentrated gut contents, they also ingest xylem sap.[19] Plant-species-dependent differences in phloem sap composition, e.g. sucrose concentration, may also provoke distinct feeding patterns in aphid species.

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